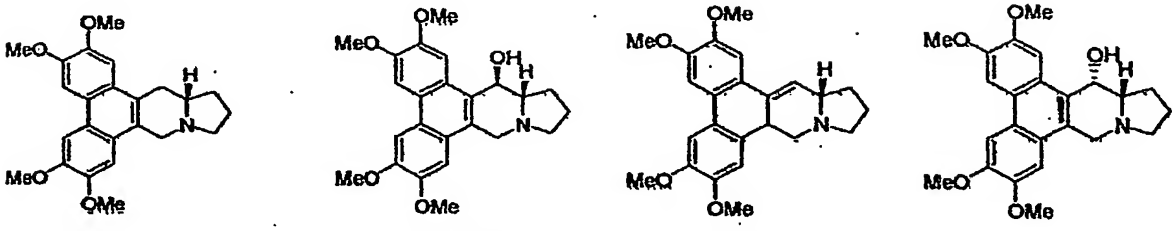
FIGURE 1

Table 1. Growth inhibition and loss of clonogenicity of KB (human nasopharyngeal carcinoma) and HepG2 (hepatocyte carcinoma) cells.

A. Chemical structure



B. EC50 (growth inhibition)

cell line	DCB-3500 (μM)	DCB-3501 (μM)	DCB-3502 (μM)	DCB-3503 (μM)
KB	0.012±0.003	0.106±0.084	0.234±0.091	0.028±0.004
HepG2	0.011±0.004	0.110±0.045	0.264±0.115	0.035±0.005

C. LD50 (colony forming ability)

cell line	DCB-3500 (μM)	DCB-3501 (μM)	DCB-3502 (μM)	DCB-3503 (μM)
KB	0.83±0.31	3.07±1.17	6.29±3.74	1.55±0.47
HepG2	0.04±0.006	0.21±0.13	0.45±0.13	0.17±0.08

FIGURE 1 CONT'D

Table 2. EC50 of DCB-3500, 3501, 3502 and 3503 on the growth inhibition of KB cells and its drug resistant cells.

A. EC50

Cell line	DCB-3500 (μM)	DCB-3501 (μM)	DCB-3502 (μM)	DCB-3503 (μM)
KB	0.012±0.003	0.106±0.084	0.234±0.091	0.028±0.004
KB-MDR	0.014±0.005	0.143±0.082	0.282±0.138	0.026±0.008
KB-7D	0.012±0.007	0.225±0.206	0.527±0.080	0.045±0.010
KB-7D-Rev	0.011±0.006	0.070±0.057	0.289±0.188	0.025±0.010
KB-Hu-R	0.025±0.007	0.070±0.042	0.218±0.167	0.036±0.006
KB-Hu-Rev	0.016±0.004	0.045±0.004	0.127±0.095	0.028±0.016
KB-100	0.020±0.010	0.103±0.033	0.179±0.063	0.038±0.001
KB-100-Rev	0.010±0.003	0.118±0.031	0.247±0.122	0.041±0.009

B. Description of resistant cell lines

Cell line	Biochemical changes	Resistant to
KB-MDR	gp 170 ↑	VP-16, Taxol, Adrimycin, Vincristine
KB-7D	Topo II ↓, MRP↑	VP-16, Vincristine, Adrimycin
KB-7D-Rev	Topo II ↓	VP-16, Adrimycin
KB-Hu-R	Ribonucleotide reductase ↑ CdR kinase ↓	Hydroxyurea, AraC, Gemcitabine
KB-Hu-Rev	CdR kinase ↓	AraC, Gemcitabine
KB-100	Topo I ↓ XRCC 1 ↑	camptothecin, topotecan, SN-38
KB-100-Rev	Topo I ↓ XRCC 1 ↑	camptothecin, topotecan, SN-38

FIGURE 1 CONT'D

Table 3. Impact of DCB-3500 and 3503 on cell cycle progression

KB		G0-G1	S	G2-M
Control	24 h	75	10	15
Nocodazole		0	10	90
3500 0.03 uM		62	19	19
3500 0.1 uM		56	26	18
3500 0.3 uM		54	30	16
3500 1 uM		52	36	12
3503 0.1 uM		68	18	14
3503 0.3 uM		52	33	15
3503 1 uM		63	24	13
3503 3 uM		57	30	13

HepG2		G0-G1	S	G2-M
Control	24 h	45	27	28
Nocodazole		2	18	80
00 0.03 uM		47	29	24
00 0.1 uM		44	33	23
00 0.3 uM		51	21	28
00 1 uM		41	27	32
03 0.1 uM		50	26	24
03 0.3 uM		55	20	25
03 1 uM		53	21	26
03 3 uM		47	25	28

FIGURE 1 A

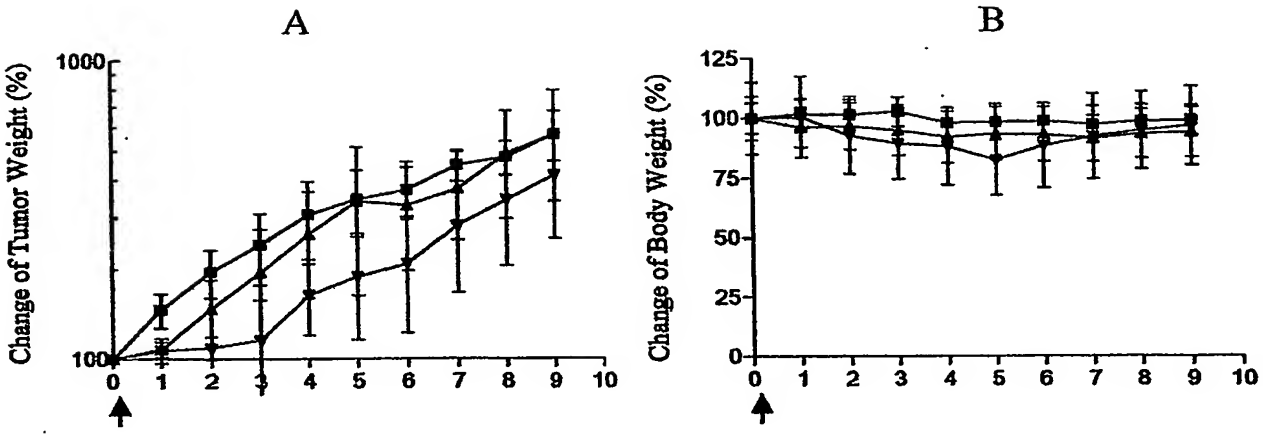

A
Change of Tumor Weight (%)
1000
100
0 1 2 3 4 5 6 7 8 9 10
B
Change of Body Weight (%)
125
100
75
50
25
0
0 1 2 3 4 5 6 7 8 9 10

FIGURE 2

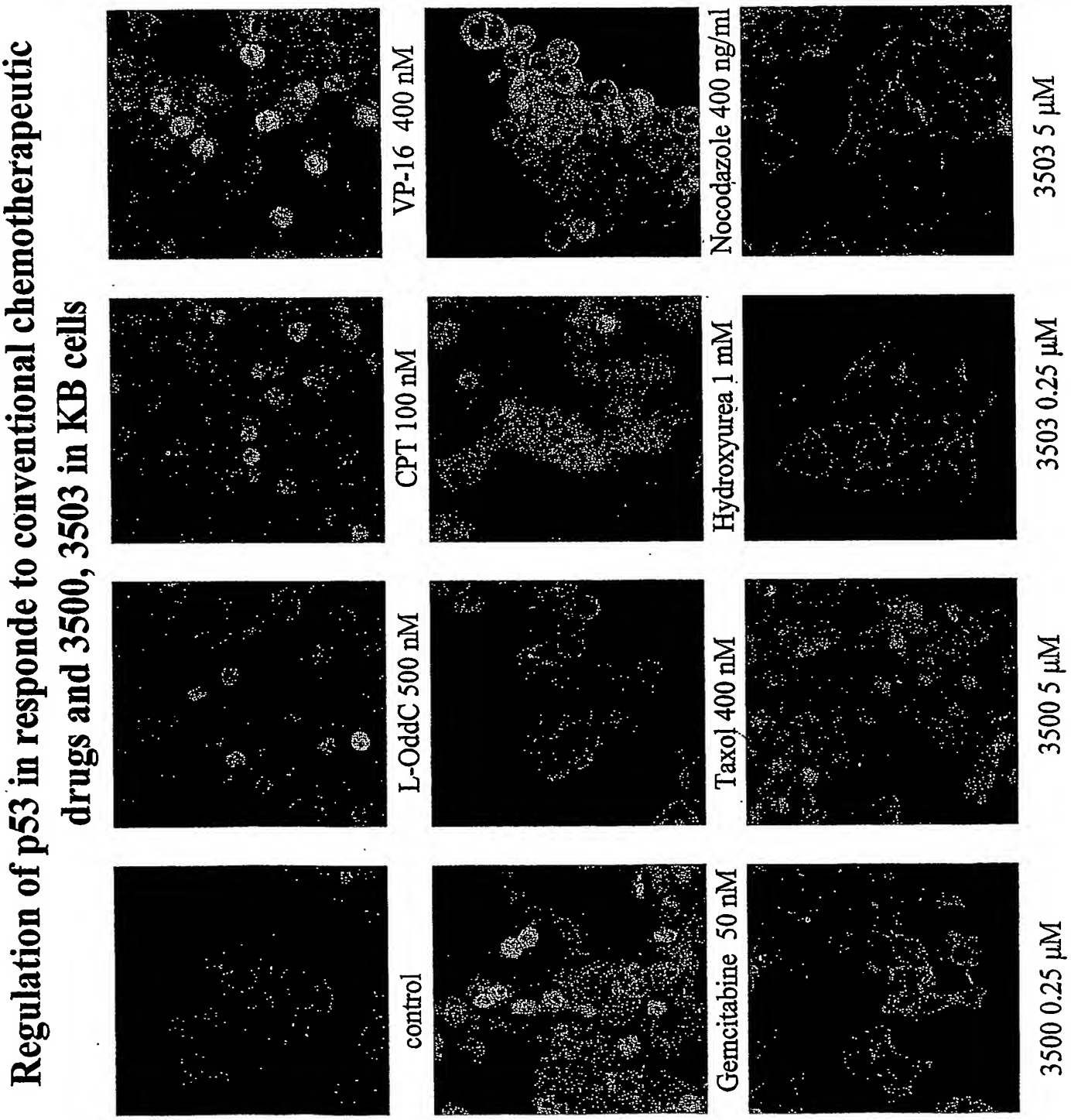
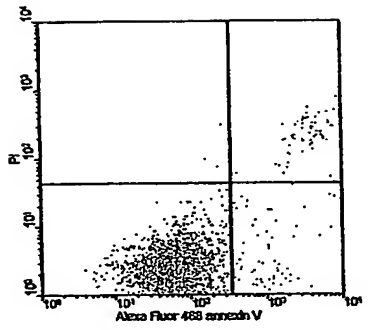
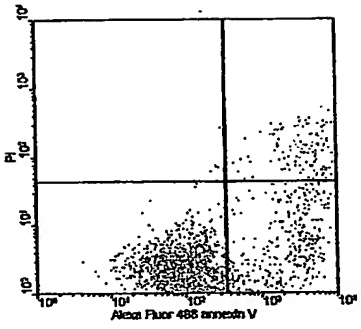

Regulation of p53 in responde to conventional chemotherapeutic drugs and 3500, 3503 in KB cells
control
L-OddC 500 nM
CPT 100 nM
VP-16 400 nM
Gemcitabine 50 nM
Taxol 400 nM
Hydroxyurea 1 mM
Nocodazole 400 ng/ml
3500 0.25 µM
3500 5 µM
3503 0.25 µM
3503 5 µM

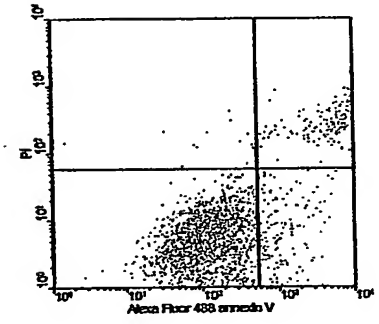
FIGURE 3



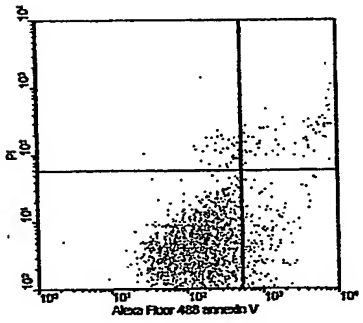
KB untreated



KB 3503 3μM



HepG2 untreated



HepG2 3503 3μM

FIGURE 4

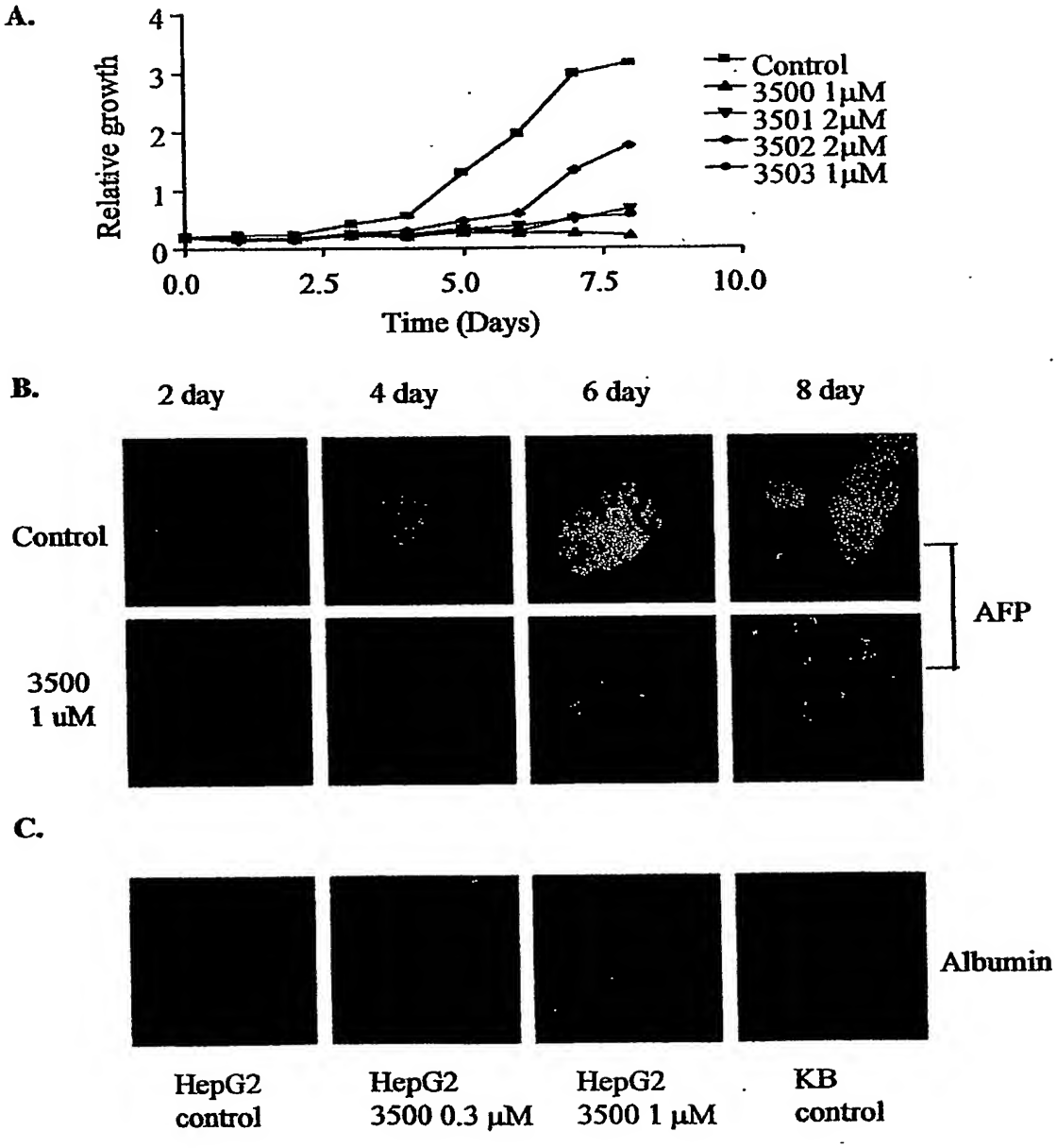
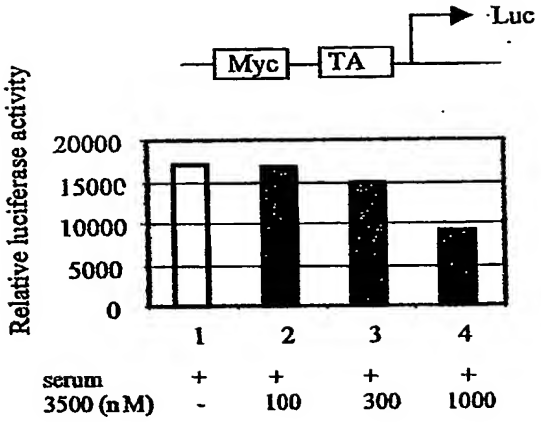

A.
Relative growth
Time (Days)
Control
3500 1μM
3501 2μM
3502 2μM
3503 1μM
B.
2 day
4 day
6 day
8 day
Control
3500
1 uM
AFP
C.
Albumin
HepG2
control
HepG2
3500 0.3 μM
HepG2
3500 1 μM
KB
control

FIGURE 5

A.


Luc
Myc
TA
Relative luciferase activity
20000
15000
10000
5000
0
1
2
3
4
serum + + + +
3500 (nM) - 100 300 1000

B.

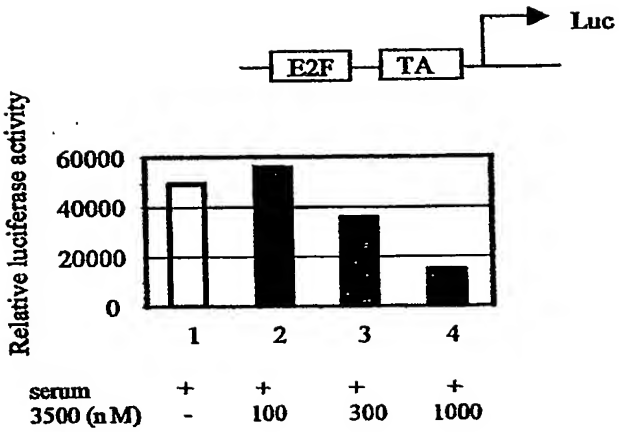
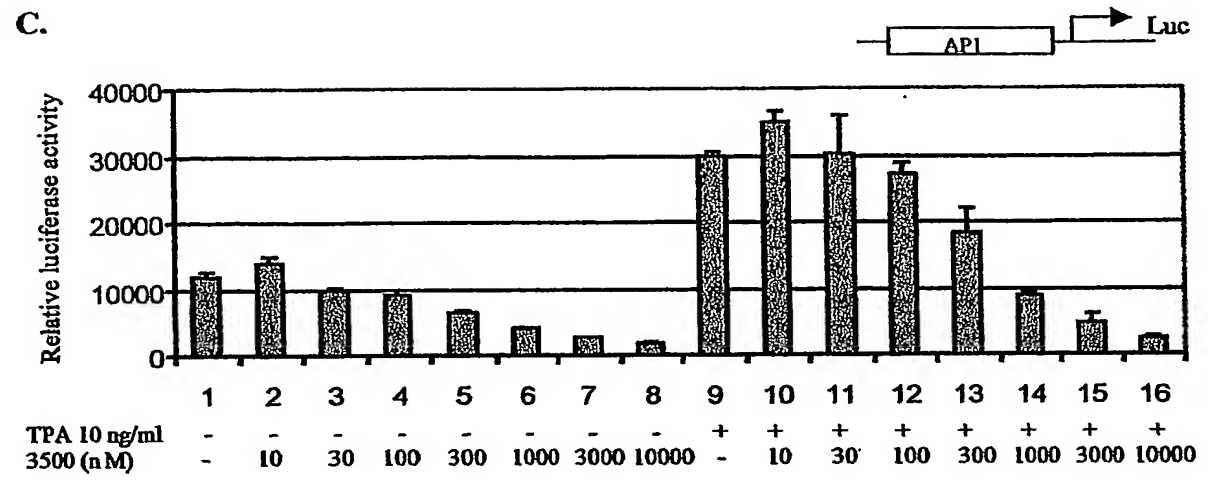
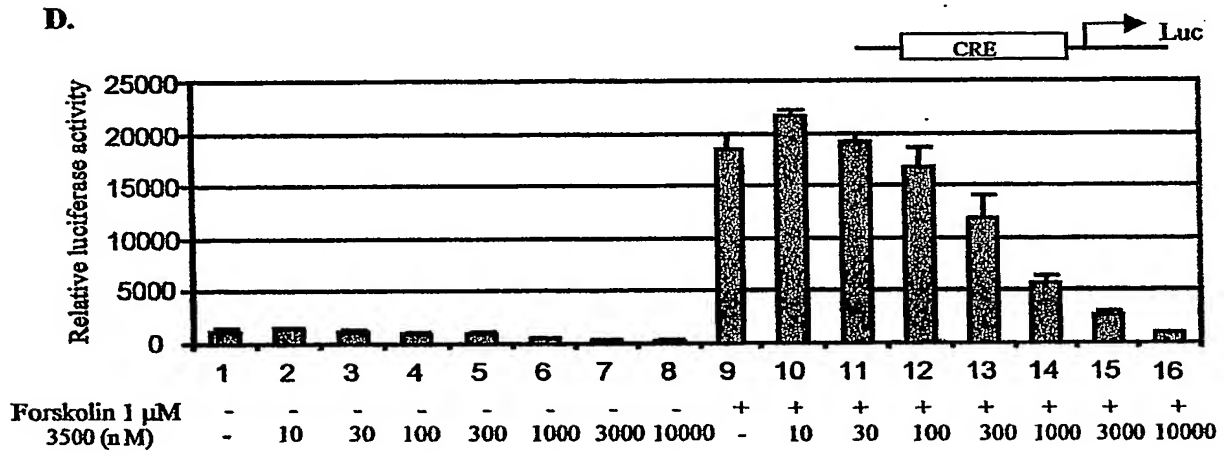

Luc
E2F
TA
Relative luciferase activity
60000
40000
20000
0
1
2
3
4
serum + + + +
3500 (nM) - 100 300 1000

FIGURE 5 CONT'D





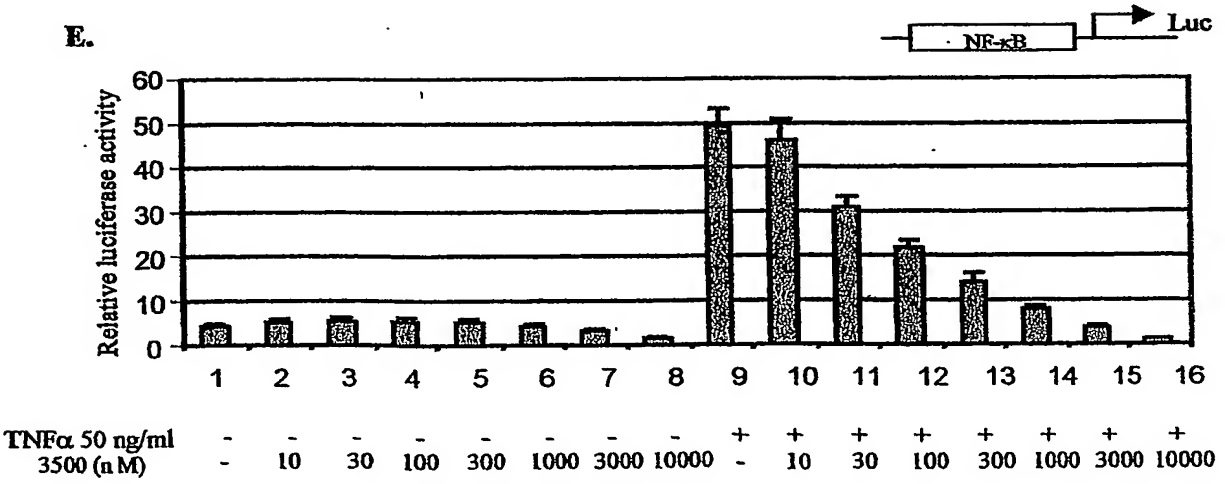
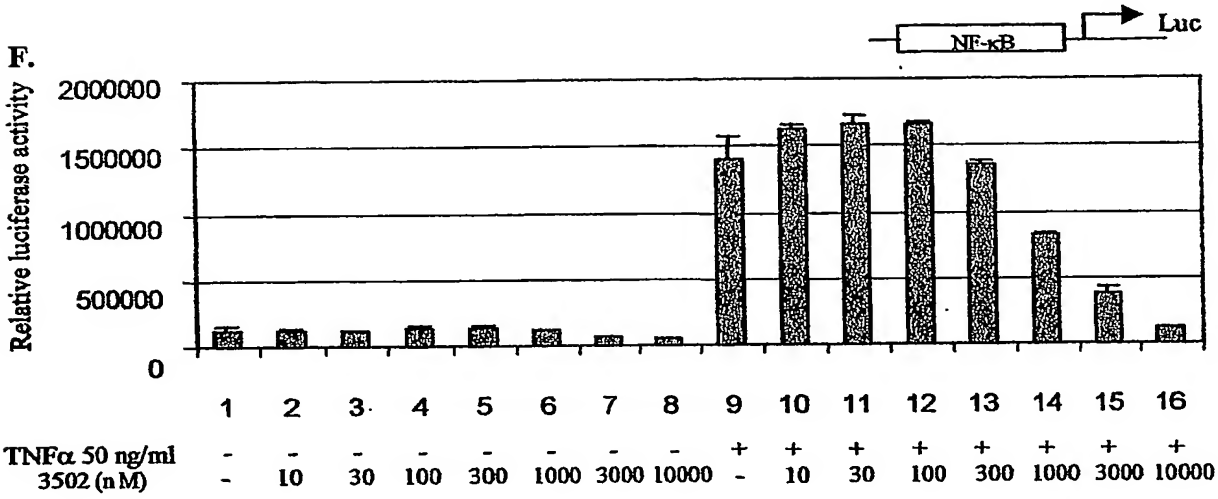


FIGURE 5 CONT'D



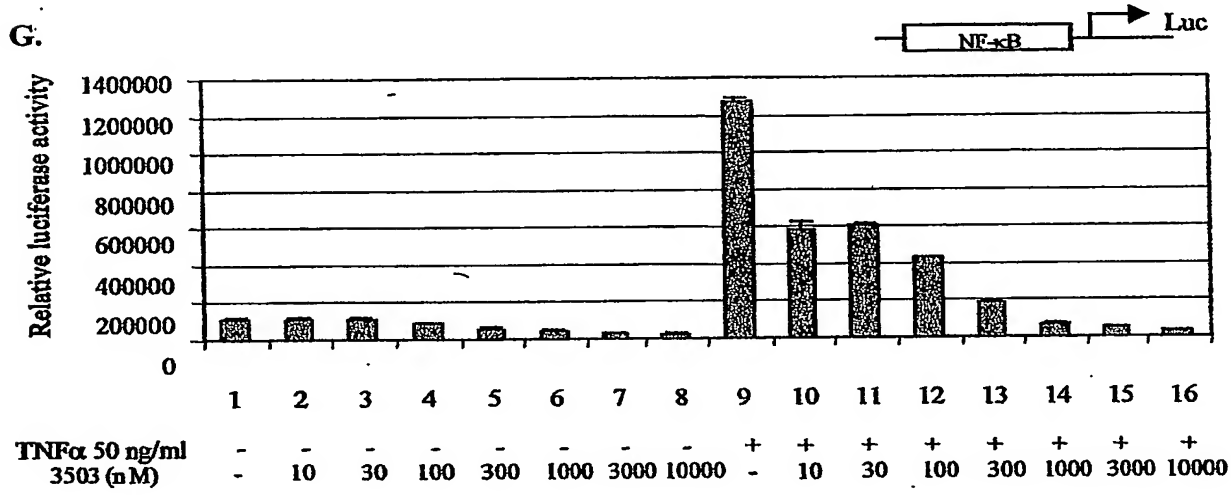


FIGURE 6

Scheme I

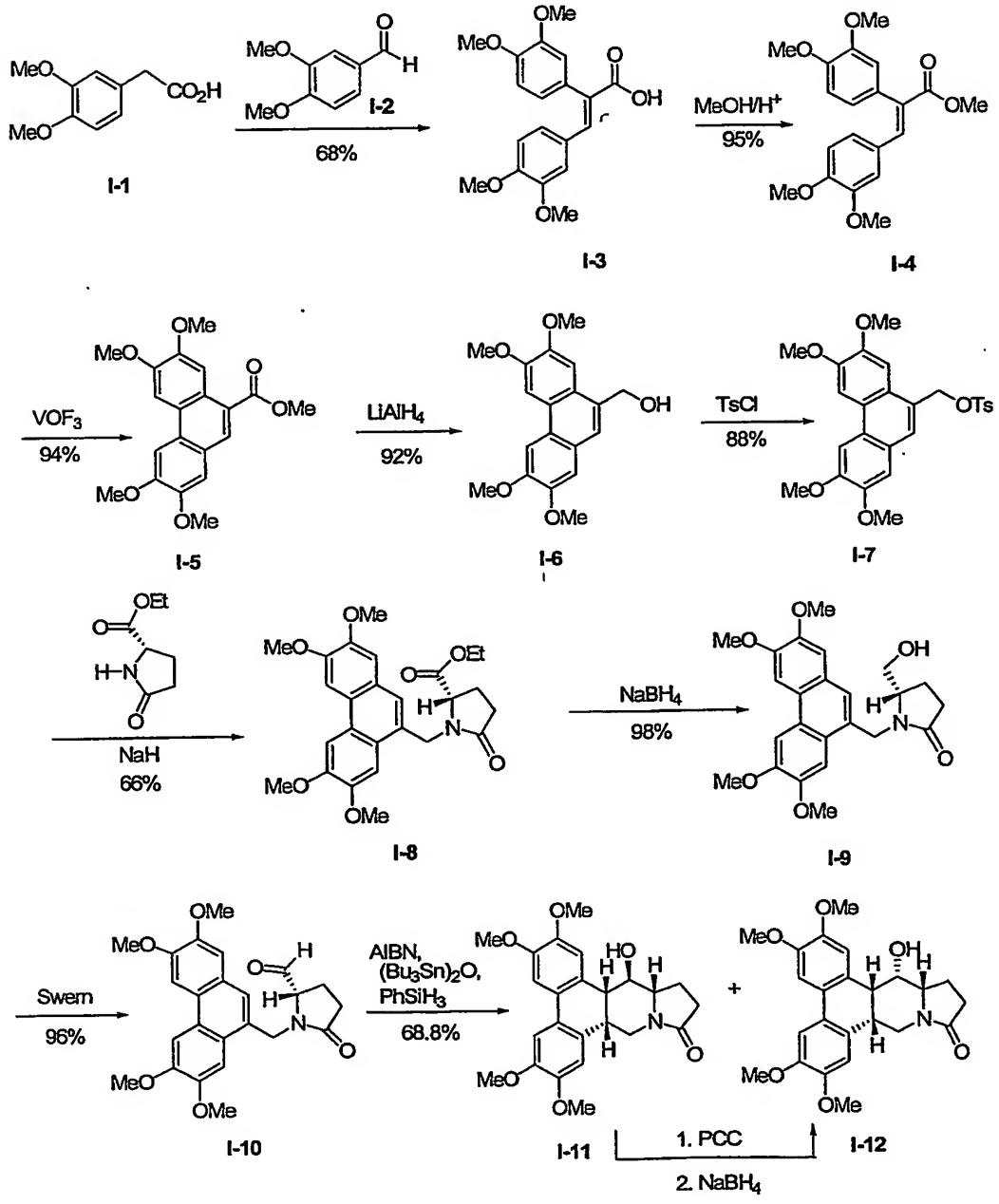


FIGURE 7

Scheme II

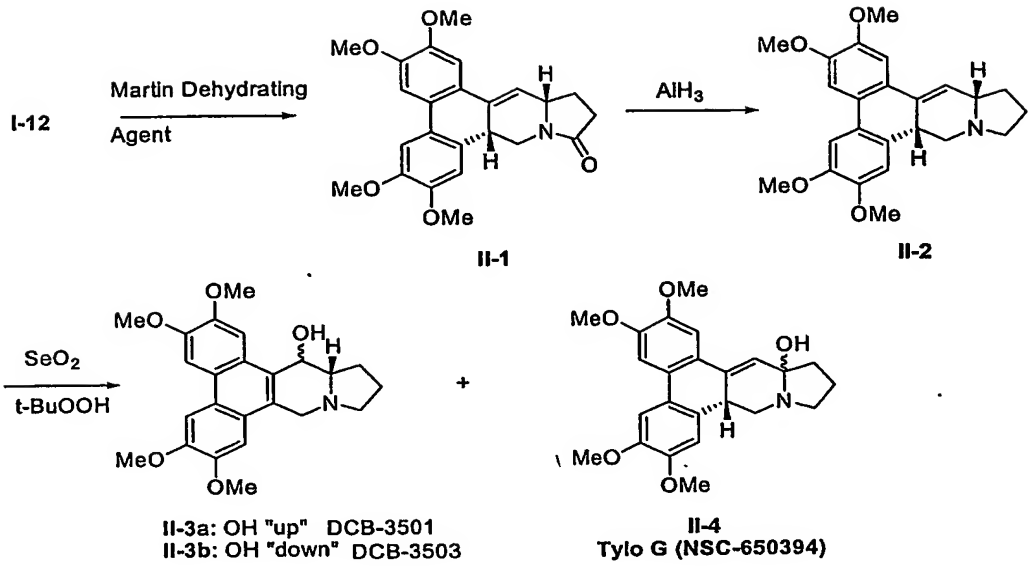


FIGURE 8

Scheme III

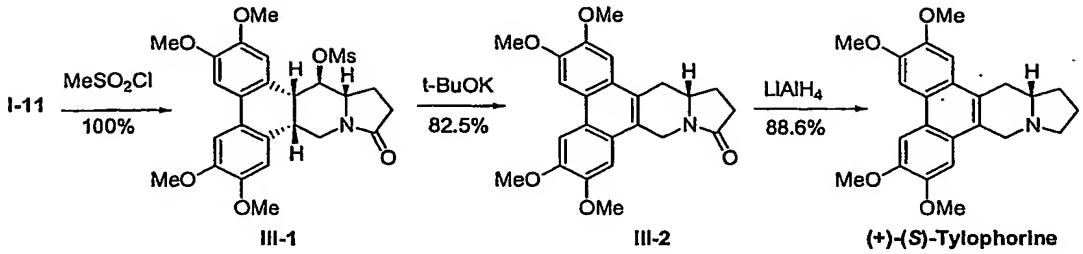


FIGURE 9

Scheme IV

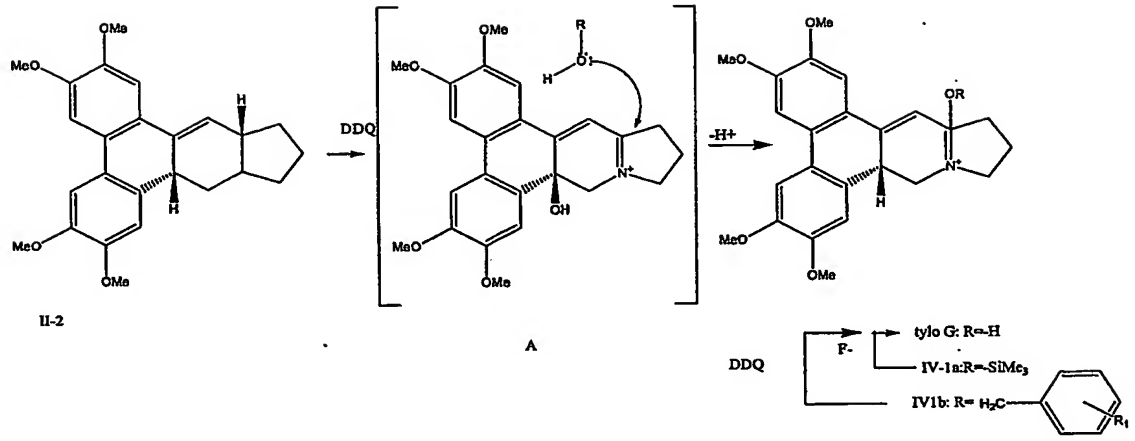


FIGURE 10

Scheme V

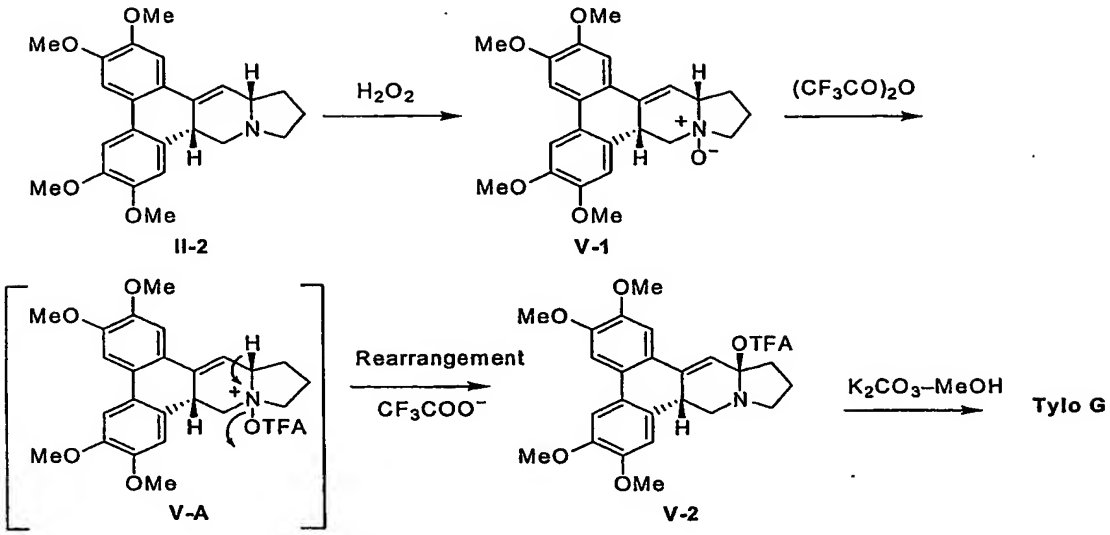


FIGURE 11

Scheme VI

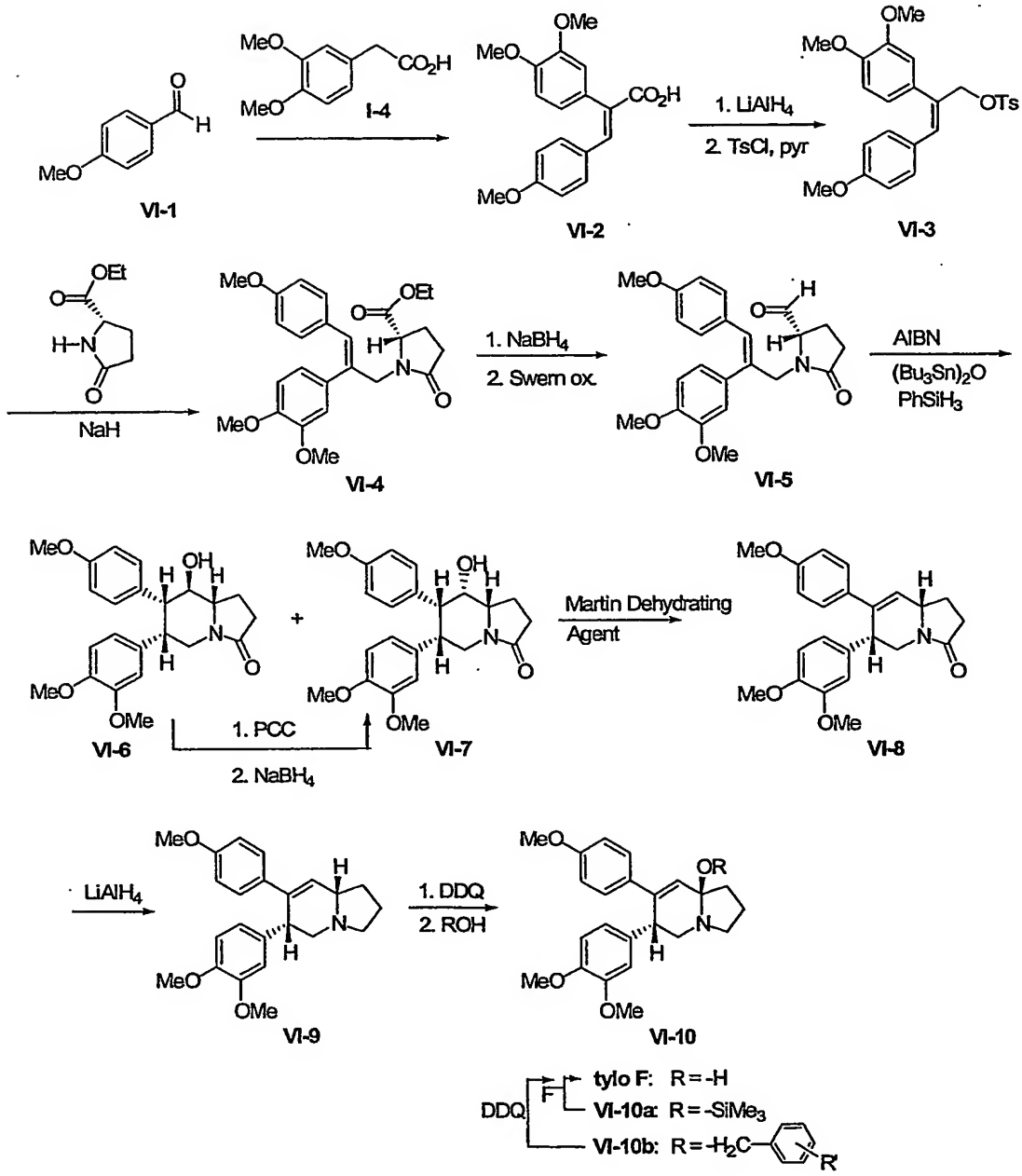


FIGURE 12

Scheme VII

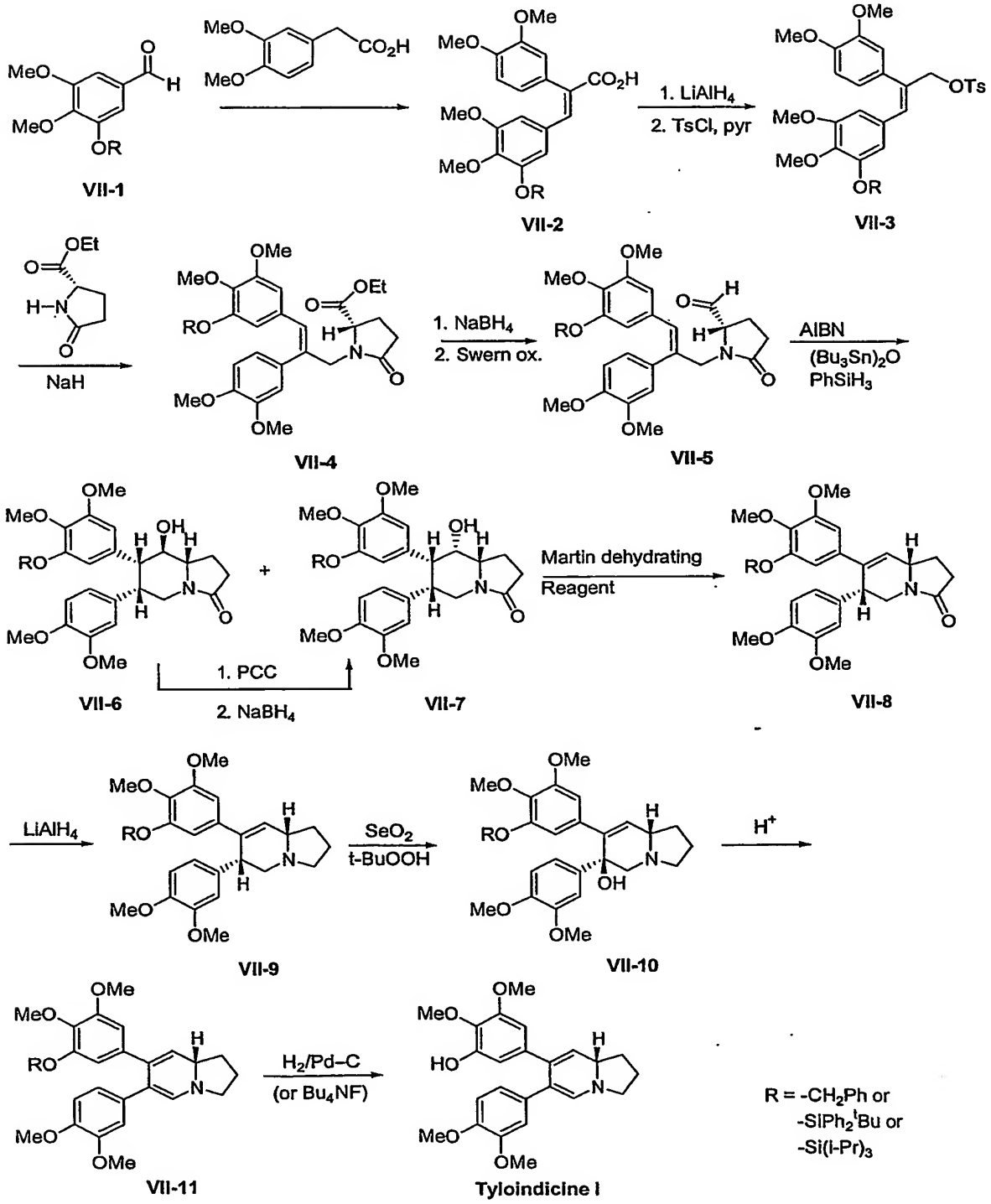


FIGURE 13

Scheme VIII

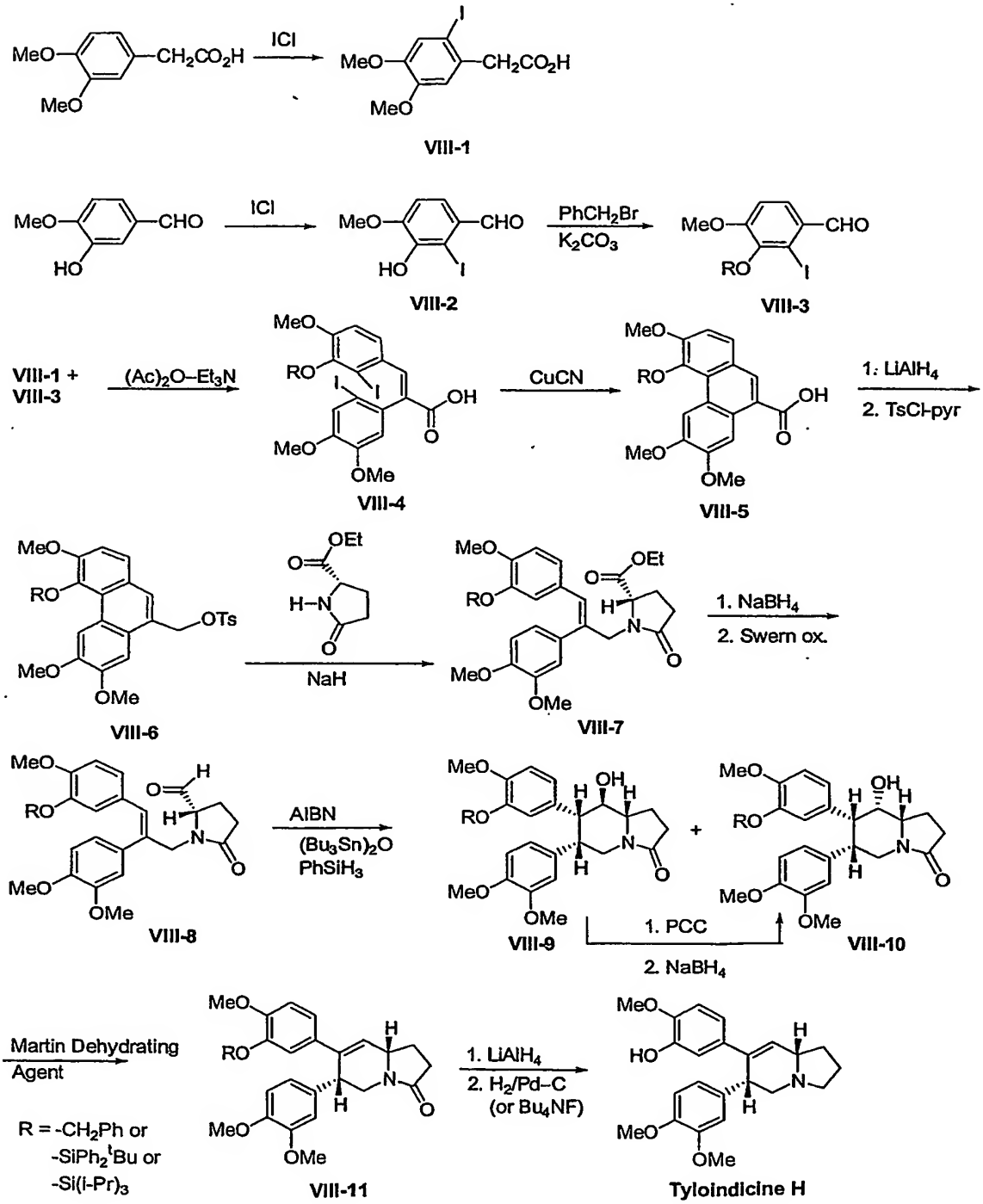
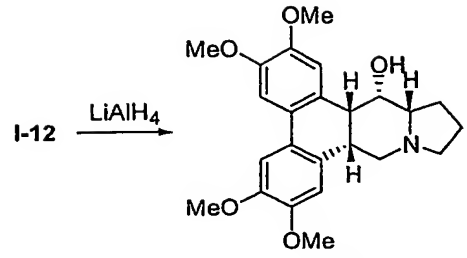


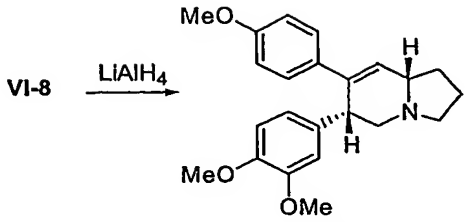
FIGURE 14

Scheme IX



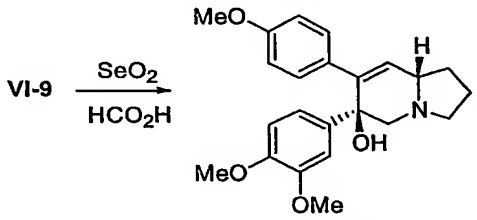
IX-1

Gives analogue (epimer) to NSC-717334. Similar conversions possible for **VI-6, VI-7, VII-6, VII7, VIII-9, VIII-10. VII-6, VII-7, VIII-9, VIII-10** can be deprotected, then reduced for additional analogues. A few will be made to search for activity.



IX-2

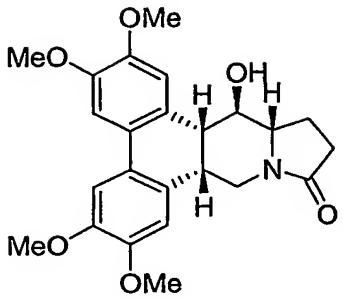
Screen **VII-9** and deprotect to give analogue.



IX-3

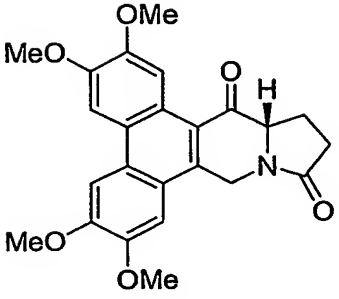
Gives the tylo F analogue of **II-3** (NSC-716802, active). **VII-10**, deprotect. Also $LiAlH_4$, SeO_2 on **VIII-11**, deprotect.

FIGURE 15



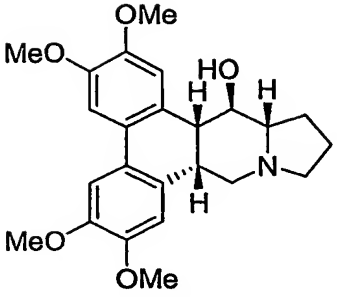
NSC-717334, **I-11**

(III)



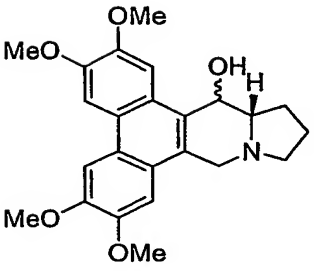
NSC-712822

(IV)



NSC-717336

(V)



II-3a: OH "up" DCB-3501
II-3b: OH "down" DCB-3503, NSC-716802

(VI)

FIGURE 16

Scheme X

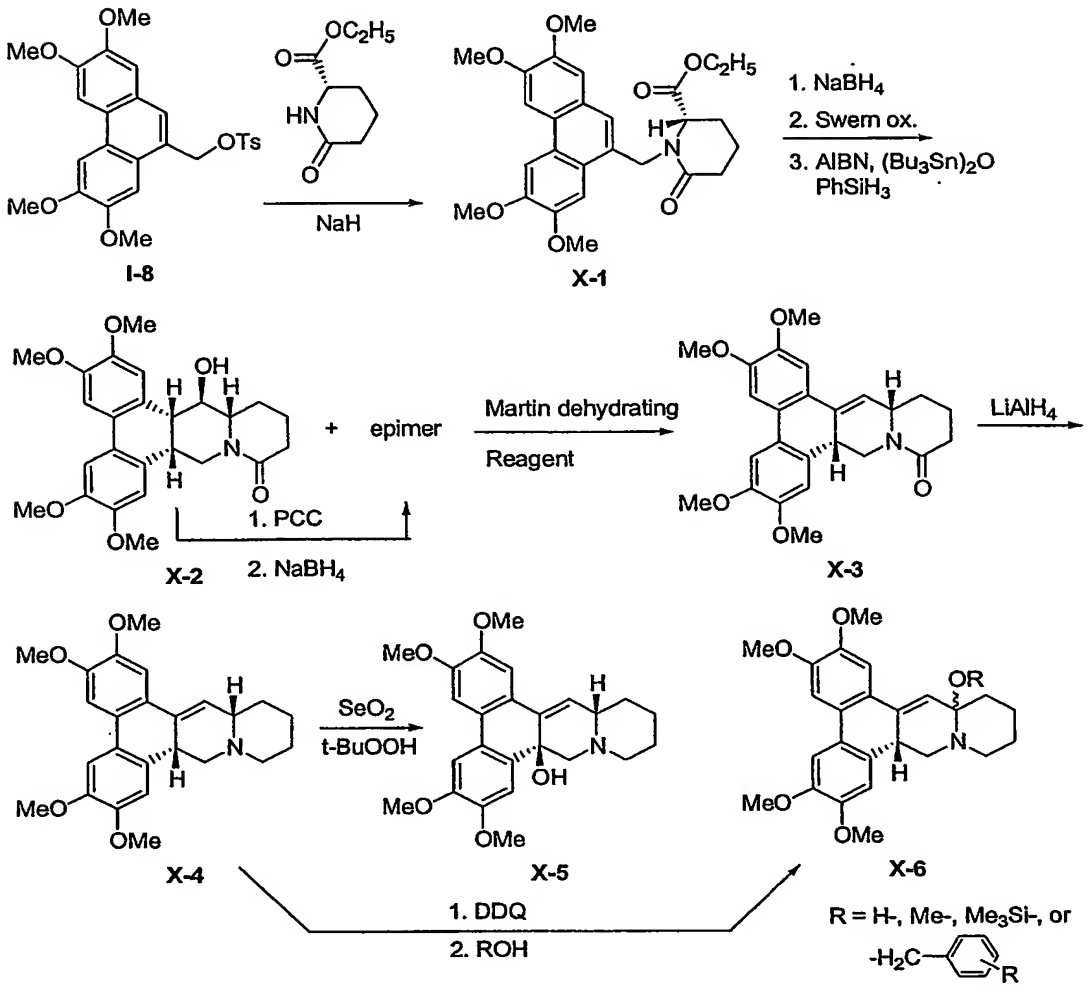


FIGURE 17

Scheme XI

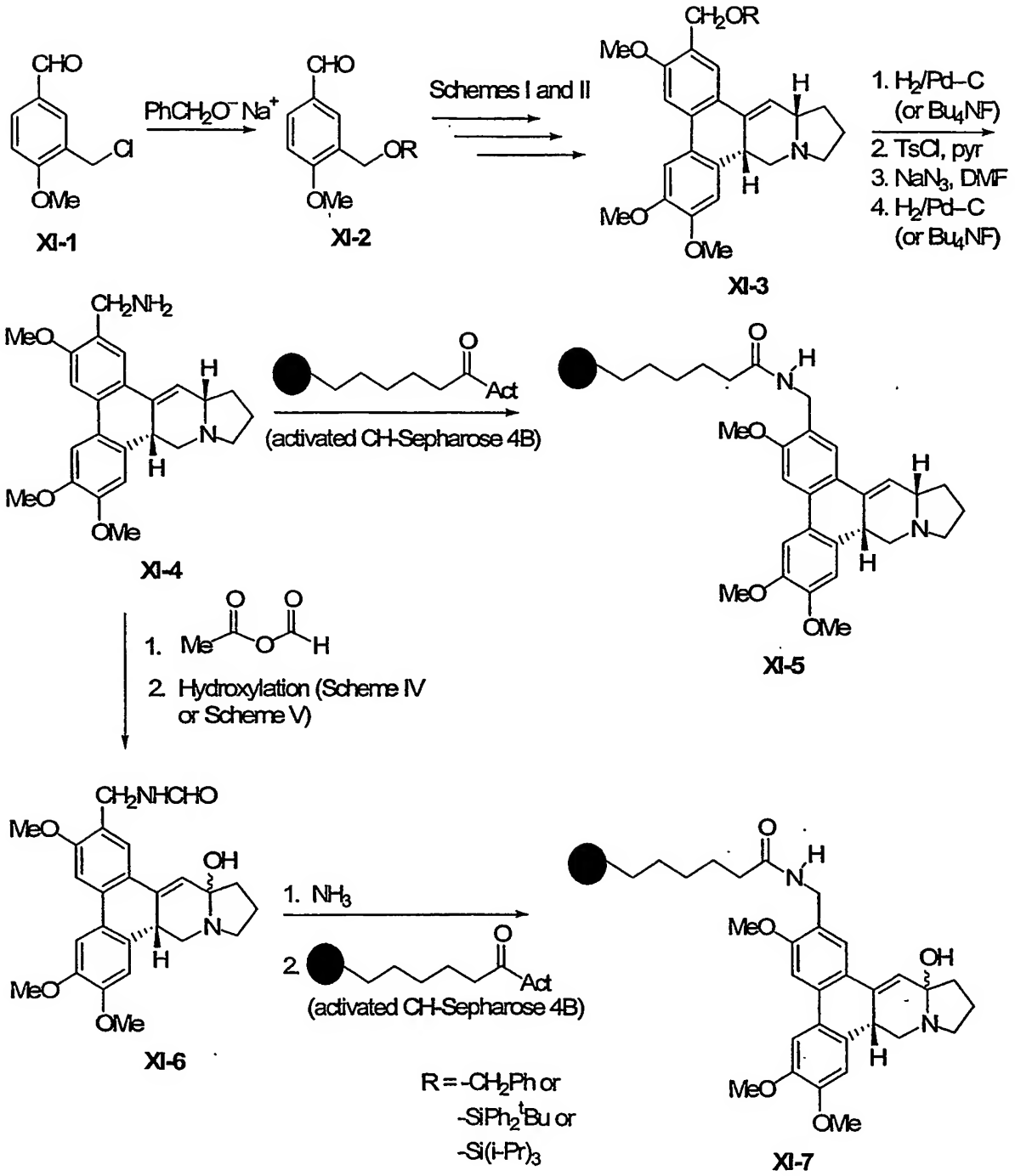


FIGURE 18

B. HepG2

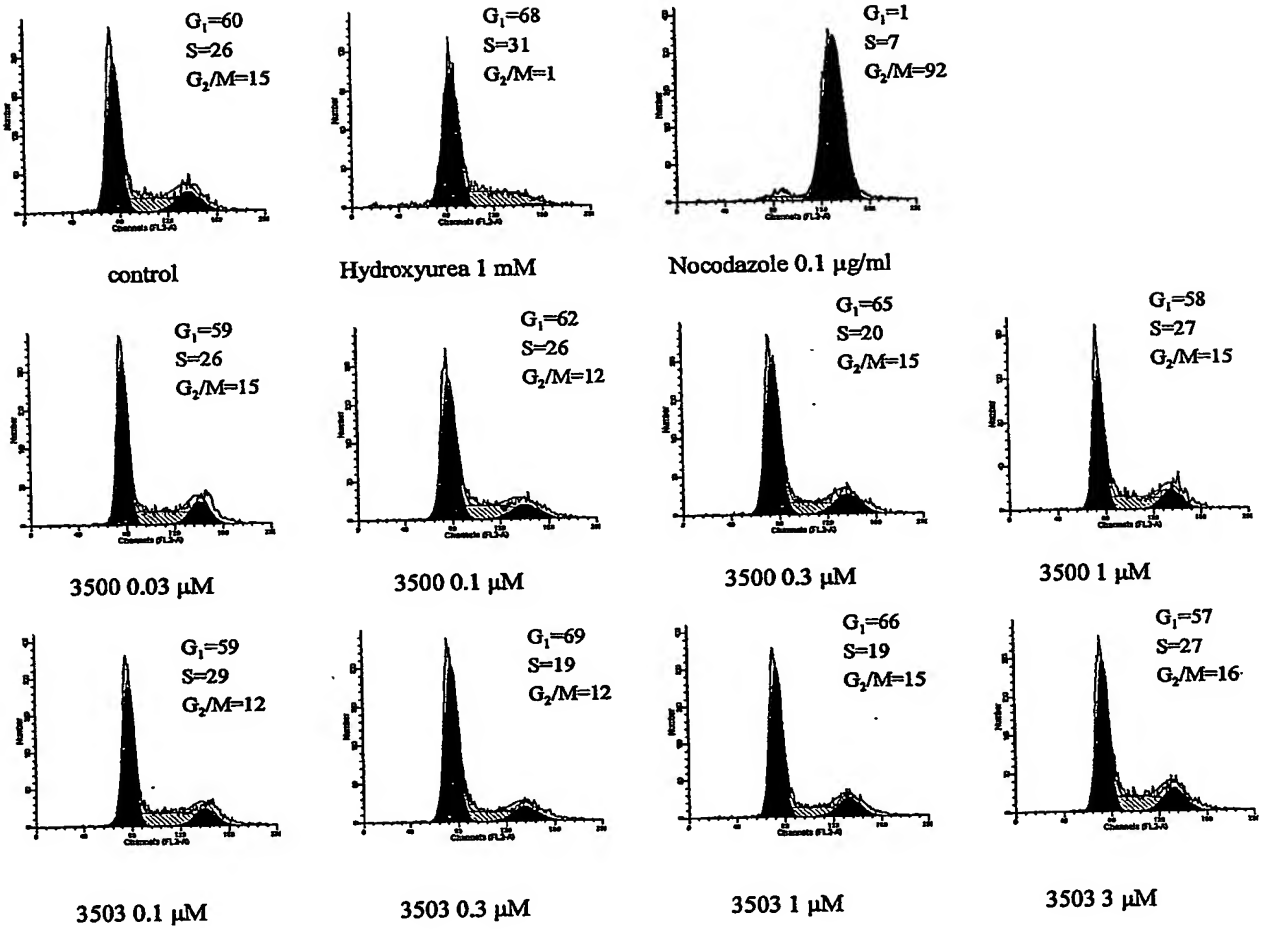


FIGURE 19

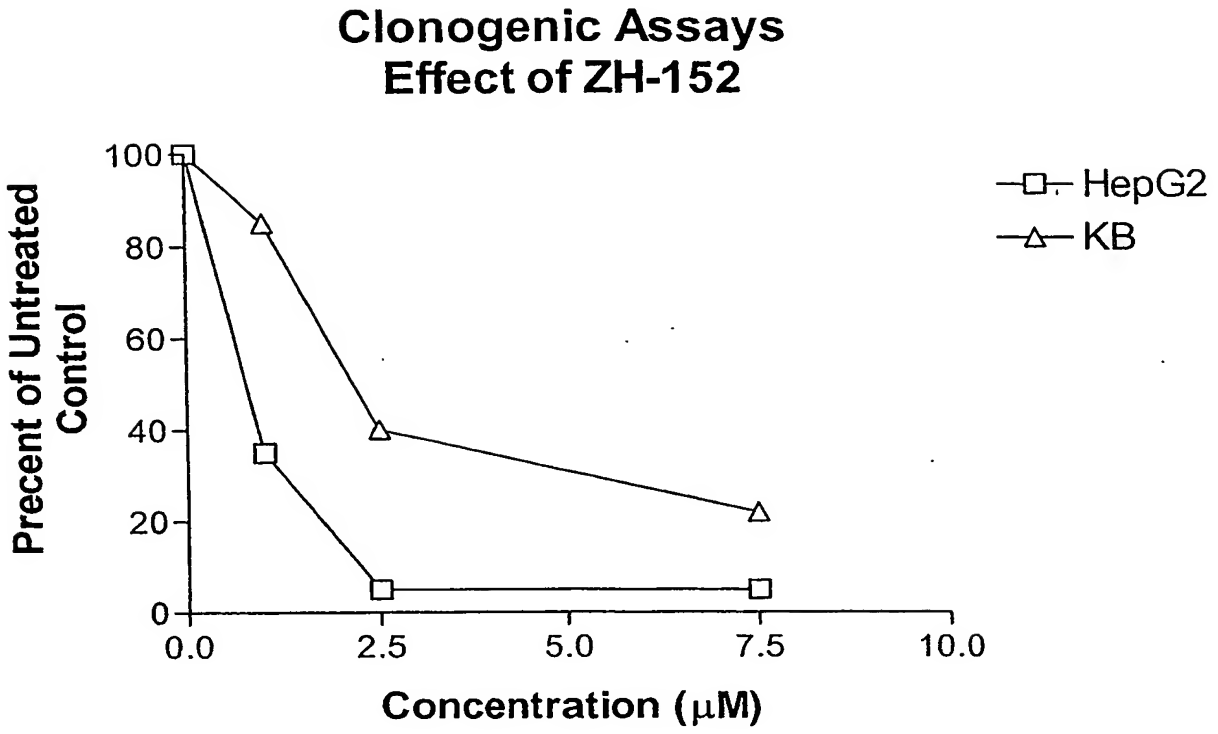
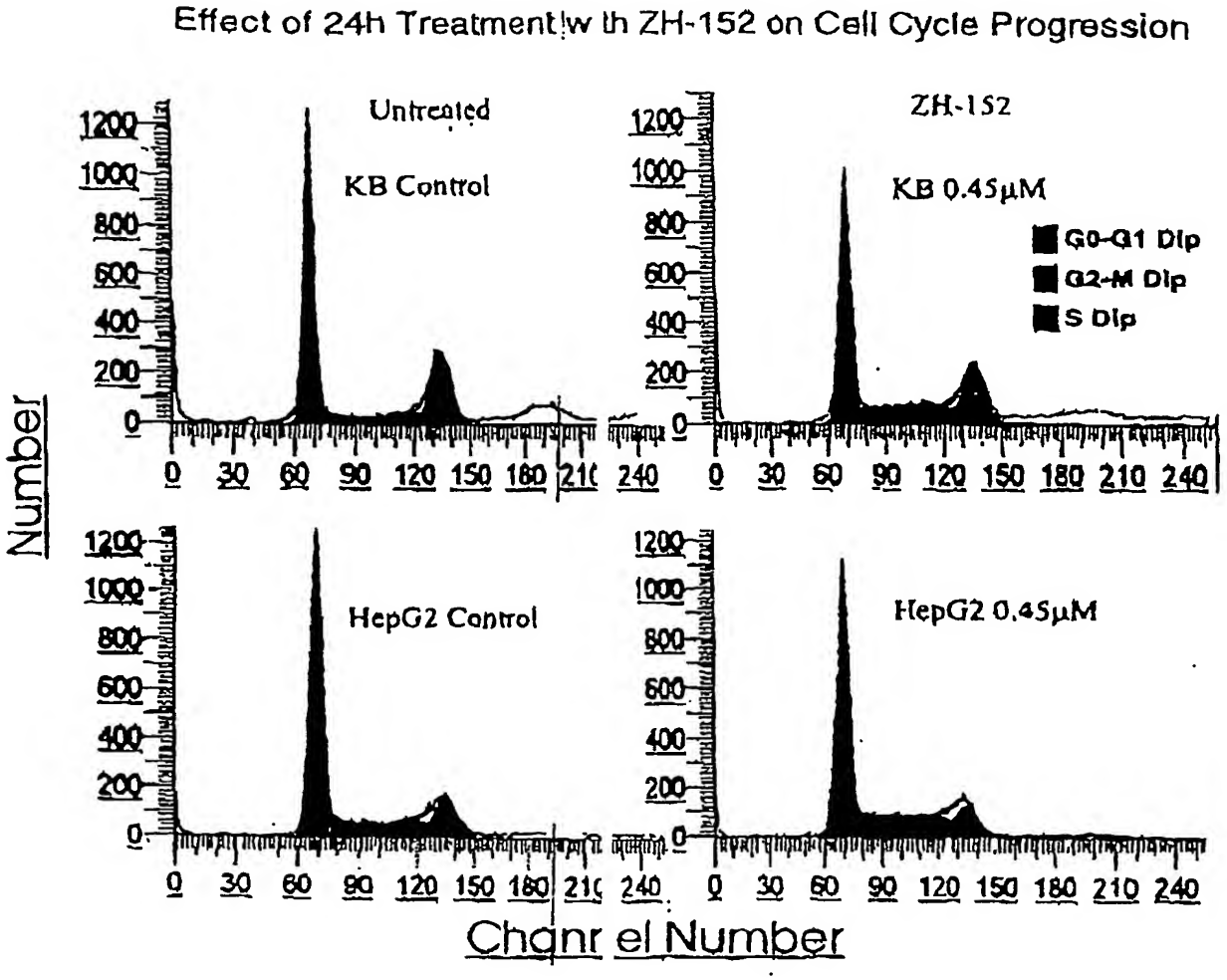

Clonogenic Assays
Effect of ZH-152
Precent of Untreated Control
100
80
60
40
20
0
0.0
2.5
5.0
7.5
10.0
Concentration (μM)
HepG2
KB

FIGURE 20



ZH-152 slows down the cell progression in S-phase of both cell lines. Thus, the growth inhibition of these two cell lines by ZH-152 is due to the inhibition at targets responsible for S-phase progression. Additional biochemical determinants may play a role in the preferential killing (loss of clonogenecity) of HepG2 to that of KB.

FIGURE 21

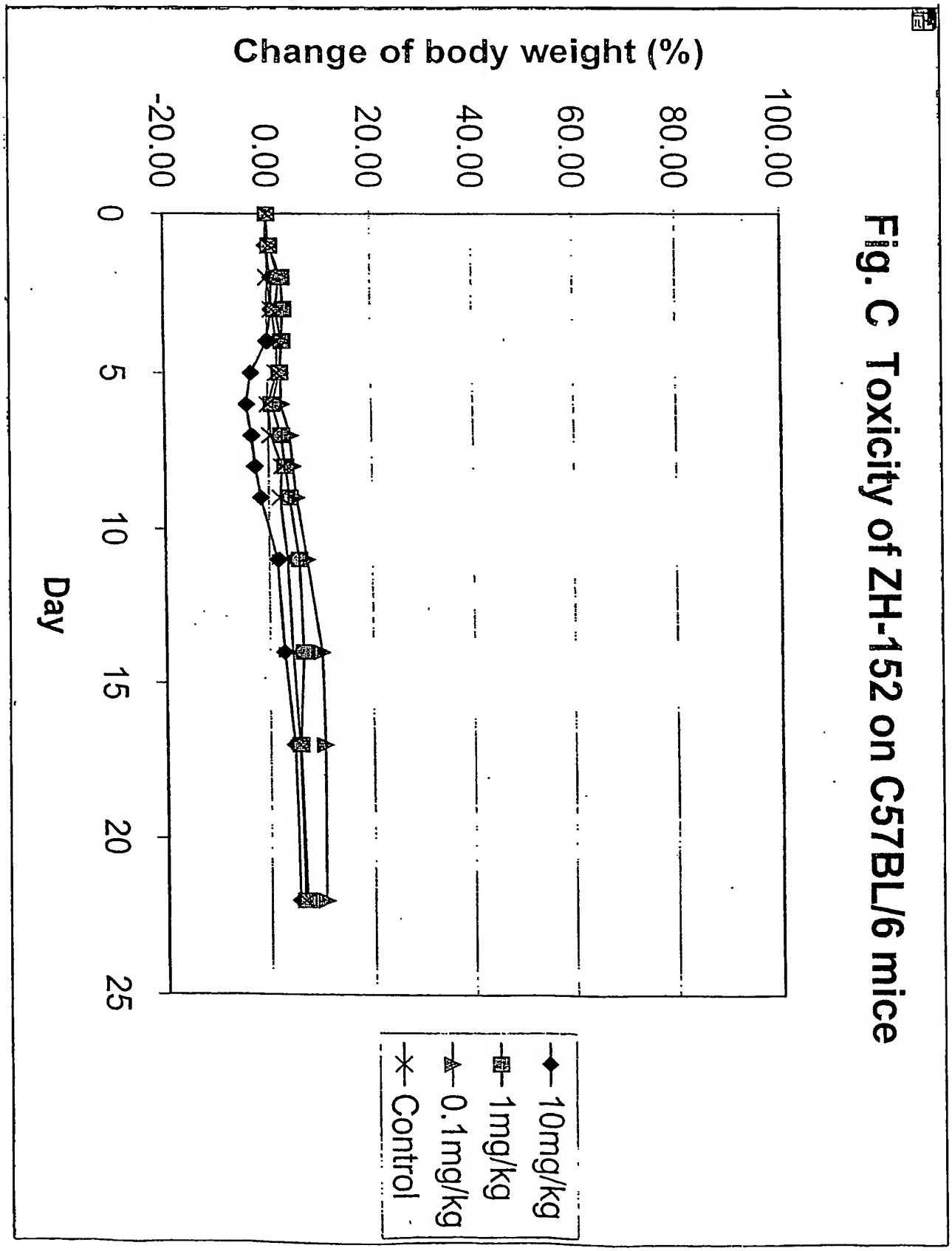


Fig. C Toxicity of ZH-152 on C57BL/6 mice

FIGURE 22

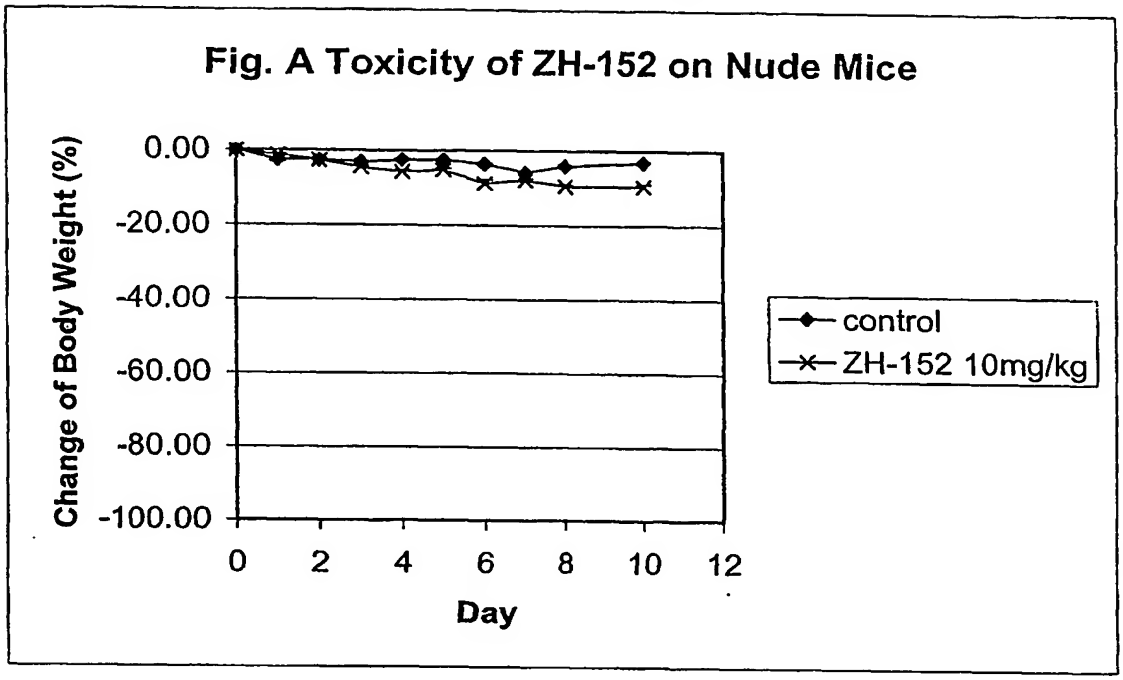


Fig. A Toxicity of ZH-152 on Nude Mice
Change of Body Weight (%)
0.00
-20.00
-40.00
-60.00
-80.00
-100.00
0
2
4
6
8
10
12
Day
control
ZH-152 10mg/kg

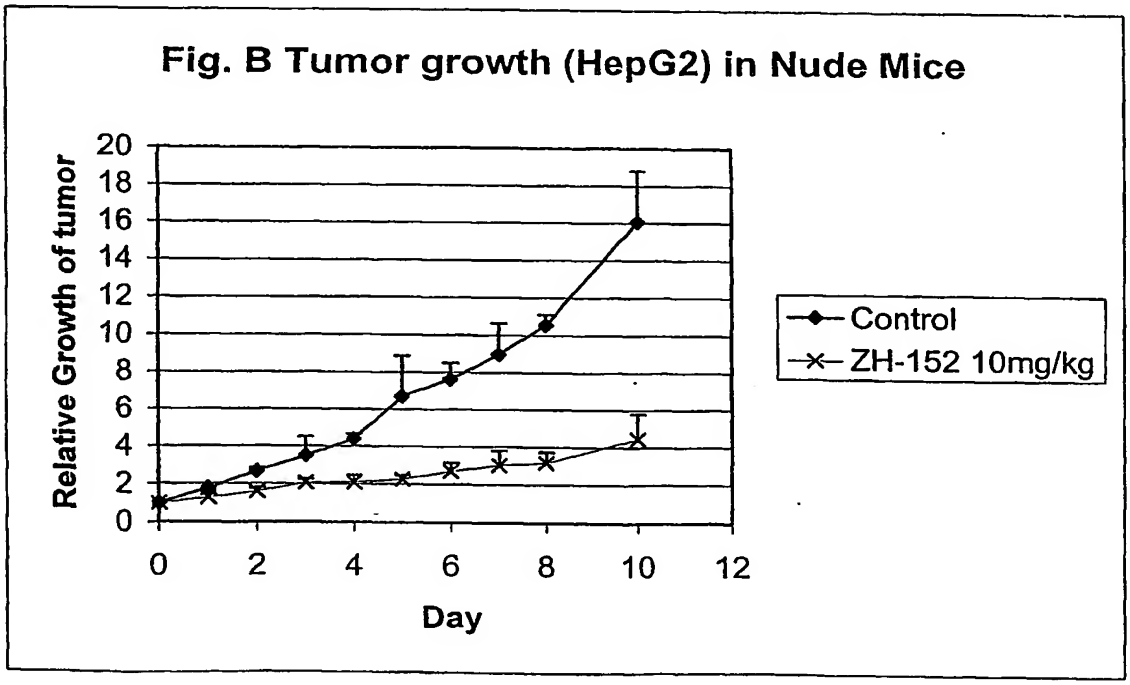


Fig. B Tumor growth (HepG2) in Nude Mice
Relative Growth of tumor
20
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
10
12
Day
Control
ZH-152 10mg/kg